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# Digestive enzymes of a sugarcane borer, *Chilotraea infuscatellus* Snell.

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**Abstract.**—The enzymes maltase, sucrase, trehalase, beta-fructosidase, chymotrypsin, aminotripeptidase, leucine aminopeptidase, prolinase, glycyl-L-leucine dipeptidase and flycyl-glycine dipeptidase were detected in the salivary glands of *C. infuscatellus*. The gut showed activity for all these in addition to beta-glucosidase, alpha-galactosidase, beta-galactosidase and trypsin. The significance of synthesis of oligosaccharides in the gut is to avoid hyperglycaemia. Out of the four distinct substrate specific alpha-glucosidases, three-(maltase, sucrase and trehalase) were present; raffinose was not hydrolysed by sucrase. Separate enzymes were involved for the hydrolysis of sucrose, raffinose and melibiose; alpha-galactosidase did not hydrolyze raffinose.

## Introduction

Although a wide variety of digestive enzymes have been reported in the Class Insecta, a detailed knowledge of the presence of peptidases in this group is still not available. The present paper gives a detailed account of the digestive enzymes found in the salivary glands, and in the contents and tissues of the three different regions of the gut of the last larval instar of *Chilotraea infuscatellus*. It also attempts to clarify the ambiguity regarding glycosidase specificity.

## Materials and methods

The larvae of *C. infuscatellus* were collected from infested sugarcanes in the vicinity of Lucknow and reared at 27°C on sliced sugarcane which were changed daily. Enzyme extracts were prepared and enzymes assayed as described previously (Agarwal, 1975 & 1976).

## Results

The results are summarised in Table I, which gives qualitative and comparative quantitative data of various enzymes. In the salivary gland sucrase, trehalase, beta-fructosidase, chymotrypsin, amino-tripeptidase, leucine aminopeptidase, prolinase, glycyl-L-leucine dipeptidase and glycyl-glycine dipeptidase, and a very weak activity of maltase were detected.

Many workers have noted the formation of oligosaccharides in the gut of insects and their presence in the excreta. Duspiva (1953), Srivastava and Auclair (1962), Yang and Davis (1968), and Ishaaya and Swirski (1970) are of opinion that oligosaccharides are produced by the action of invertase on sucrose. It has been further demonstrated that oligosaccharides can also be synthesized in presence of melezitose, melibiose (Srivastave, 1966), and maltose (Takanona and Hori, 1974). Gilmour (1961) and Dadd (1970) regarded that the synthesis of oligosaccharides is due to transglycolysation reaction of alpha-glucosidases. On this basis Gilmour (1961) regarded invertase as a sugar-transferring enzyme and also thus recognized two types of (functionally different) (Dadd, 1970) alpha-glucosidases in insects. In *C. infuscatellus* the synthesis of oligosaccharide is due to reverse catalysis by some glycosidase's when glucose alone or glucose and fructose both are produced or present in high concentrations in the gut, as has been pointed out in *Sesamia inferens* (Agarwal, 1976). Further the chromatograms of Srivastava and Auclair (1962) further support these observations although their conclusions were different.

The synthesis of oligosaccharides in an insect seems to be an important measure in limiting the diffusion of monosaccharides in the gut when present in excess and are not required by the insect. Thus the insect is able to avoid hyperglycaemia and ultimately excretes the oligosaccharide to avoid its hydrolysis again. Such a feedback mechanism enables the insect to feed on a variety of diets having different percentages of digestable carbohydrates. The sugarcane borer larva can feed on sugarcane throughout the year, during which period the percentage of sucrose varies widely in its sugarcane foodplant.

Gilmour (1961) did not include trehalase among the digestive enzymes of insects. Bursell (1970) has pointed out that in insects trehalase is capable of catalysing the synthesis of trehalose, but this pathway is probably not of physiological significance. Trehalose is found in a high concentration in the haemolymph of insects, but is generally absent in their normal food. Thus some of its may diffuse into their gut (Wyatt, 1967), but when there is a shortage of carbohydrates in the diet the loss may affect their survival. Thus trehalase in the gut of insects may be a necessity to avoid such loss, because as soon as trehalose would diffuse from the haemolymph in the gut, it would be broken into glucose which would be resorbed (by diffusion gradiant) as glucose is practcally absent in the haemolymph. Trehalase thus functions as a digestive enzyme.

*C. infuscatellus* has three distinct substrate specific alpha-glucosidase (maltase, sucrase and trehalase), observations which support my previous report (Agarwal, 1976) where it has been clearly demonstrated that in insects instead of a generalized alpha-glucosidase there are at least four distinct substrate specific alpha-glucosidases.

Weidenhagen's (1932) conservative view regarding the specificity of glycosidases has been supported by various workers. Fraenkel (1940), Krishna and Saxena (1962), Khan and Ford (1962 and 1967), Banks (1963) and Evans and Payne (1964) are of opinion that alpha-galactosidase which hydrolyzes melibiose may hydrolyze raffinose also and that beta-fructosidase which hydrolyzes raffinose may also hydrolyze sucrose. Dadd (1970), and Takanona and Hori (1974) doubted the hydrolysis of sucrose by beta-fructosidase, but recognized the concept of the hydrolysis of raffinose by alpha-galactosidase. The confusion regarding the specificity of alpha-galactosidase and beta-fructosidase continued probably because distinct situations were not detected in any insect. In *C. infuscatellus* (Table II) the midgut tissue hydrolyzed melibiose and sucrose and did not hydrolyze raffinose, this means that raffinose is not hydrolyzed either by sucrase or by alpha-galactosidase. The midgut contents hydrolyzed melibiose, sucrose and raffinose, an observation which establishes the presence of beta-fructosidase. Beta-fructosidase attacks raffinose between fructose and melibiose only, as is evident from the hydrolytic products of raffinose digestion by the salivary glands, foregut contents and hindgut contents.

Sugarcane contains a varity of free amino acids (Bhattacharya and Mukherjee, 1953) of which leucine, lysine and valine are regarded as essential. Three other essential amino acids (arginine, histidine and isoleucine) (Singh and Singh, 1964) are also provided by the hydrolysis of sugarcane proteins. Thus the larva gets only six essential amino acids from its diet. The remaining four essential amino acids (methionine, phenylalanine, threonine and tryptophan) (Wigglesworth, 1965; Dadd, 1970) may be regarded as non-essential or dispensable essential or if needed by the larva may be obtained by the transaminase system's generally present. In its haemolymph only phenylalanine was detected (Rakshpal and Singh, 1976) out of these four. This supports the suggestion that methionine, threonine and tryptohan may not be essential for the larva and it is capable of synthesizing phenylalanine.

In the foregut tissue only trehalase was detected, while the gut contents revealed all the enzymes of the salivary glands. The activity of matase, chymotrypsin and glycyl-L-leucine dipeptidase was very weak, however.

The midgut tissue showed activity of maltase, sucrase, trehalase, beta-glucosidase, alpha-galactosidase, beta-galactosidase, trypsin, chymotrypsin, aminotripeptidase and glycyl-glycine dipeptidase. Midgut contents showed a strong activity of all these in addition to beta-fructosidase, leucine aminopeptidase, prolinase and glycyl-L-leucine dipeptidase.

The hindgut tissue also gave evidence of the activity of sucrase, trehalase, chymotrypsin, aminotripeptidase and glycyl-L-leucine dipeptidase; while its contents showed the presence of all these enzymes along with beta-glucosidase, prolinase and glycyl-glycine dipeptidase; and a very weak activity of beta-galactosidase, beta-fructosidase, trypsin and leucine aminopeptidase.

When the sucrose contents were high in the diet of the larva, synthesis of a oligosaccharide took place in its gut which was excreted, but when the sucrose contents were low this synthesis was not observed. *In vitro* studies confirmed that the oligosaccharide synthesis took place when a high concentration of sucrose was taken as substrate.

When raffinose was hydrolyzed by the salivary glands, foregut contents and hindgut contents, melibiose and fructose were produced.

**Discussion**

*C. infuscatellus* feeds on sugarcane which contains a very high percentage of sucrose besides some glucose and fructose. Sucrose is therefore the most important many other carbohydrates, namely maltose, lactose, melibiose, trehalose and raffinose. For the hydrolysis of these metabolites, glycosidases are present in its gut. However, none of these carbohydrates are detected in sugarcane. Sucrase may therefore, be regarded as an obligatory enzyme while the other glycosidases are facultative.

The activity of sucrase is high throughout the gut of the larva, and its diet contains high percentage of sucrose. This relationship between sucrase and sucrose clearly supports the view of House (1965), Wigglesworth (1965) and Dadd (1970) that when in an insect there is high activity of any enzyme its substrate must be present in abundance in the diet.

At present little is known about the different aspects of proteases in insects. Now it can only be stated that in insects protein digestion takes place in three stages to librate amino acids, the raw materials essential for growth and maintenance of tissue proteins.

In *C. infuscatellus* some enzymes from the midgut did not reach the hindgut; suggesting that they are either denatured, digested, or self-hydrolyzed in the posterior part of the midgut. Similarly some enzymes of the hindgut contents were not detected either in hindgut tissue or in the excreta, which suggests denaturing, digestion or self-hydrolysis in the Posterior part of the hindgut. The Presence of some enymes in both hindgut tissue and contents, but their absence or low activity in the excreta, may be due to absorption in the hindgut (Agarwal, 1976). This would appear to be a conservative measure to retain as much enzyme protein as possible.

In the salivary glands of *C. infuscatellus* a large variety of enzymes were detected, all of which enter the foregut lumen along with the food. Thus the digestion of the food starts in the foregut as soon as the saliva is mixed with food-stuff. It has been claimed that the enzymes from the midgut can reach the foregut. In *C. infuscatellus* the enzymes from the midgut did not reach the foregut, since the enzymes exclusively of the midgut were not detected in the foregut. Further, antiperistalsis was never observed among the hundreds of larvae dissected. It is interesting to note that some enzymes are secreted only by the salivary glands and that they reach as far back as the hindgut via foregut and midgut.

From the proportionate hydrolysis in the three regions of the gut of the larva it is evident that the major part of digestion takes place in the midgut. The presence of a wide varity of enzymes in the gut normally not required may suggest its adaptive capacity, enabling survival under adverse nutritional conditions.

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## TABLE I

**Digestive enzymes in salivary glands and various gut regions of *Chilotraea infuscatellus***

| Enzyme tested | Salivary glands | Foregut T | Foregut C | Midgut T | Midgut C | Hindgut T | Hindgut C |
|---|---|---|---|---|---|---|---|
| I. Carbohydrases | | | | | | | |
| 1. Amylase (3.2.1.1) | − | − | − | − | − | − | − |
| 2. Cellulase (3.2.1.4) | − | − | − | − | − | − | − |
| 3. Alpha-glucosidases | | | | | | | |
| i. Maltase (3.2.1.20) | | − | | + | ++ | − | − |
| ii. Melezitase | − | − | − | − | − | − | − |
| iii. Sucrase (3.2.1.26) | +++ | − | + | +++ | +++ | + | ++ |
| iv. Trehalase (3.2.1.28) | ++ | + | ++ | ++ | +++ | + | ++ |
| 4. Beta-glucosidase (3.2.1.21) | − | − | − | + | ++ | − | + |
| 5. Alpha-galactosidase (3.2.1.22) | − | − | − | + | ++ | − | − |
| 6. Beta-glactosidase (3.2.1.23) | − | − | − | + | ++ | − | |
| 7. Beta-fructosidase (3.2.1.80) | ++ | − | + | − | + | − | |
| II. Proteases | | | | | | | |
| 1. Aminopeptidases | | | | | | | |
| i. Leucine aminopeptidase (3.4.1.1) | ++ | − | + | − | +++ | − | |
| ii. Aminotripeptidase (3.4.1.3) | ++ | − | + | | +++ | + | + |
| 2. Carboxypeptidase (3.4.2.1) | − | − | − | − | − | − | − |
| 3. Dipeptidases | | | | | | | |
| i. Glycyl-glycine dipeptidase (3.4.3.1) | + | − | + | + | ++ | − | + |
| ii. Glycyl-L-leucine dipeptidase (3.4.3.2) | ++ | − | | − | ++ | + | + |
| iii. Prolinase (3.4.3.6) | + | − | + | − | ++ | − | + |
| iv. Prolidase (3.4.3.7) | − | − | − | − | − | − | − |
| 4. Peptide peptidohydrolases | | | | | | | |
| i. Trypsin (3.4.4.4) | − | − | − | + | ++ | − | |
| ii. Chymotrypsin (3.4.4.5) | ++ | − | | + | + | + | + |

**T – Tissue; C– Contents; –Activity absent; Very weak activity; + Weak activity; ++ Moderate activity and +++ High activity.**

**TABLE II**
**Hydrolysis of three glycosides in salivary glands and different gut regions of** *Chilotraea infuscatellus*

| Enzyme source | Glycoside hydrolyzed | Glycoside not hydrolyzed |
|---|---|---|
| Salivary glands | Raffinose and Sucrose. | Melibiose. |
| Foregut tissue | — | Melibiose, raffinose and sucrose |
| Foregut contents | Raffinose and sucrose. | Melibiose |
| Midgut tissue | Melibiose and sucrose. | Raffinose. |
| Midgut contents | Melibiose, raffinose and sucrose. | — |
| Hindgut tissue | Sucrose. | Melibiose and raffinose. |
| Hindgut contents | Raffinose and sucrose. | Melibiose. |